

# Capillary Electrophoresis Immunoassay for 2,4-D

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## Abstract

A capillary electrophoresis (CE) immunoassay format for 2,4-dichlorophenoxyacetic acid (2,4-D) was demonstrated. A fluorescent labeled 2,4-D analog competes with the analyte of interest for a finite number of binding sites provided by anti-2,4-D monoclonal antibodies. CE then provides a means of separating and measuring both the free and antibody-bound fluorescent tracer using laser-induced fluorescence detection. For this assay format, the amount of free tracer is a sensitive indicator for the concentration of analyte present in the sample. A sequential injection format allows the rapid analysis of a small number of samples. The dynamic concentration range for 2,4-D in either buffer or river water is 5 ppb to 1000 ppb.

## Introduction

Methods for the rapid and cost-effective detection of pesticides in environmental settings have gained considerable attention in recent years, primarily due to concerns related to potential human exposure from spray run off and spills into ground water sources. Laboratory-based methods for analysis of pesticides such as 2,4-D include GC, HPLC, and CE. These methods often require extensive extraction and derivitization which can increase the time and expense associated with the analysis. The use of immunoassay techniques has resulted in a wide variety of relatively rapid and inexpensive screening methods for pesticides and other environmental pollutants. Immunoassay methods reported for 2,4-D include ELISA, agglutination, fluorescence polarization, and fluorescence microbead assays as well as several antibody-based biosensor methods. Even though these methods can be sensitive and specific, there are certain advantages that can be realized by coupling the high specificity of immunofluorescence techniques with the high resolution of separation techniques such as CE. In this report, we describe the coupling of immunofluorescence to capillary zone electrophoresis with laser-induced fluorescence detection. This assay is characterized with respect to analysis of 2,4-D in laboratory buffer and spiked river water.

## Summary & Conclusions

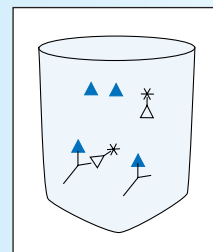
A variety of immunoassay methods have been reported for the detection and measurement of 2,4-D, each with distinct analytical and assay format characteristics. The dynamic concentration range for this assay (i.e., 5-1000 ppb) is similar to that reported for fluorescence polarization assays but is somewhat higher than ELISA or fluorescence microbead assays. One of the advantages, however, for use of CE immunoassay for environmental screening involves the relatively simple assay format and small sample volumes required (e.g., 1-60  $\mu$ L sample added a 100  $\mu$ L reaction mixture). In addition, because CE rapidly separates the bound and free analyte tracer, further development of the CE immunoassay technique may allow for multianalyte analysis in a single run and the use of less expensive and more readily available unpurified polyclonal antisera (as opposed to monoclonal antibodies).

## Notice

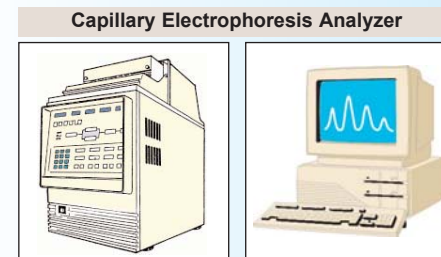
The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded the following work. Mention of trade names or commercial products does not constitute endorsement or recommendation by the U.S. EPA.

## Methods

### Assay Schematic

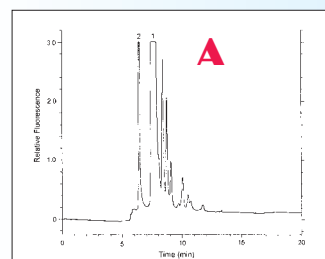


Buffer or River Water  
Y Anti-2,4-D  
\* FITC-2,4-D (tracer)  
▲ 2,4-D (analyte)  
(100  $\mu$ L Reaction Volume)

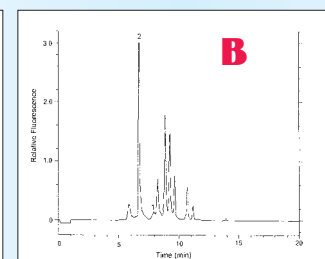


The CE immunoassay uses a direct competitive format. Among the mixture of FITC derivatives is an analyte tracer consisting of fluorescein isothiocyanate (FITC) linked to 2,4-D through an ethylenediamine linker. At constant analyte tracer concentrations, 2,4-D competes for available binding sites on the antibodies. The amount of free tracer is proportional to the amount of analyte (2,4-D) present. After incubation of the antibody, analyte, and tracer (for 1 hr), the mixture was filtered through a 0.2  $\mu$ m nylon filter and analyzed using a Beckman PACE system with laser induced fluorescence detection.

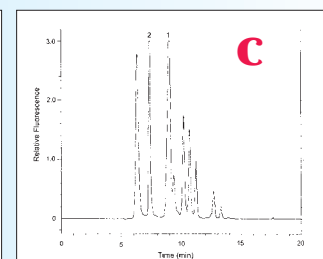
### Representative Tracings



Electropherogram A shows the analyte tracer.



Electropherogram B shows the tracer and anti-2,4-D antibody.



Electropherogram C shows the tracer, the antibody, and the analyte (2,4-D) at 1 ppm.

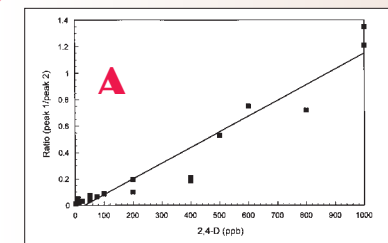
As is frequently reported for the synthesis of FITC tracers such as FITC-2,4-D, a number of fluorescent derivatives are produced. One or more of these derivatives may show affinity for the antibody. We identified a tracer component (peak #1) that competes with 2,4-D for binding sites on the monoclonal antibody E4C2. This antibody was a gift from Dr. M. Franek of the Czech Republic.

Peak #1 decreased dramatically in the presence of anti-2,4-D antibody and then increased with increasing concentrations of 2,4-D added to the reaction mixture.

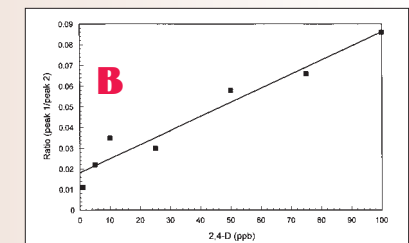
Peak #2 was insensitive to antibody or added 2,4-D. Consequently, this peak was used to normalize the assay response for variations in injection volumes.

## Results

### Calibration Plots (in Buffer)



The calibration plot (A) for 2,4-D in phosphate buffered saline (PBS, pH 7.4) was linear over a concentration range from 5-1000 ppb. Regression analysis gave an  $r^2$  value of 0.91.



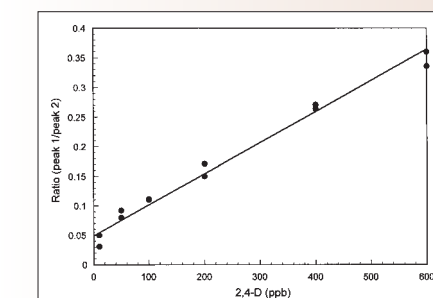
Plot B shows the calibration for 2,4-D at concentrations between 5-100 ppb. The  $r^2$  value is 0.95. The lowest amount of 2,4-D detected in the reaction mixture was 1 ng.

The CE analysis conditions were as follows:

Running Buffer; 10 mM borate, pH 9.5  
Current; 6.6  $\mu$ A  
Injection Time; 10s

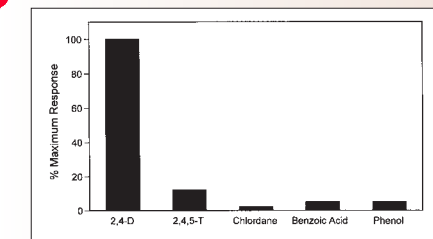
Potential; 10KV  
Temperature; 25  $^{\circ}$ C

### Calibration Plot (in River Water)



This assay was performed using ground water spiked with 2,4-D. The reaction mixture contained only antibody, analyte tracer and 2,4-D. The only sample preparation procedure prior to analysis by CE was filtration to remove particulates. The assay response was linear between 10-600 ppb. Regression analysis yielded an  $r^2$  value for this plot is 0.99.

### Cross-Reactivity Profile



The CE immunoassay showed little cross-reactivity for structurally related compounds such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and benzoic acid or structurally unrelated compounds such as chlordane and phenol (each compared at 100 ppb).